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Additional Evidence for the Existence of a Precursor Molecule of the Prothrombin Complex in Oral Anticoagulation

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In vitamin K deficiency or intake of oral anticoagulants (vitamin K-antagonists) the synthesis of the blood clotting factors of the prothrombin complex is blocked in one of its last steps. Polypeptide chain synthesis on the ribosomal level (7) is not hampered, and the demonstration of precursor products, which hypothetically could resemble the clotting factors, has been attempted in several investigations (2, 3, 5, 8). Immunochemical methods enabled Ganrot and Niléhn (2) to demonstrate two populations of prothrombin molecules in coumarin plasma, and Josso et al. (8) found an excess of immunologically demonstrable prothrombin over prothrombin activity. The present report concerns the demonstration of a precursor molecule that resembles factor IX in its antigenic properties.

Methods

Factor IX (PTC) assays were carried out with a one-stage kaolin-phospholipid activated principle (14). Clotting times were read automatically with coagulometers (DEPEX, de Bilt, The Netherlands). The PTC-inhibitor plasma was kindly supplied by Dr. Harold Roberts of Chapel Hill. The inhibitor plasma was received on 27 July 1969 and stored at -80°C . It was used in a dilution of 1:2 in the method for measuring PTC-inhibitor neutralizing capacity as described by Roberts et al. (1, 12). The titre of the inhibitor did not alter during more than a year of storage.

Results

In Table 1 it can be seen that a coumarin plasma, in this case obtained from a patient (No. 7) with virtually no factor IX activity in the plasma due to suicidal intake of the long-acting coumarin-congener phenprocoumon, contains a large amount of PTC-inhibitor neutralizing material. This particular plasma also had less than 1% factor II, VII, and X activity. Another patient (No. 8), with 8.5% factor IX activity (Thrombotest 300 sec due to overdosage of phenprocoumon), showed 22% PTC-INC (PTC Inhibitor Neutralizing Capacity). BaSO_4 adsorption removes not only "clottable" factor IX (No. 11) but also PTC-INC (No. 10) from oxalated plasma. $\text{Al}(\text{OH})_3$ has the same action on citrated plasma. PTC-INC is not mimicked by the presence of phenprocoumon, as can be judged from the result obtained with plasma No. 12, which was composed of sample No. 6 to which phenprocoumon was added *in vitro* in a concentration of 10 $\mu\text{g}/\text{ml}$. This concentration is comparable to the concentration of the coumarin-congener in samples 7 and 8 (about 7 $\mu\text{g}/\text{ml}$), assayed according to Seiler and Duckert (13).

Table 1. PTC-inhibitor neutralizing capacity (PTC-INC) of various plasma samples.

Material	Factor IX activity (%)	Expected PTC-INC (residual factor IX activity in %)	Observed PTC-INC	Number of assays	More PTC-INC than factor IX activity
1. Normal plasma	100	≤55	47	6	—
2. Diluted normal plasma	50	≤27.5	22.5	5	—
3. Diluted normal plasma	25	≤13.75	9	2	—
4. Buffer	0	<5	<5	3	—
5. B _M -plasma	<1	25–60	31	2	+
6. B-plasma	<1	¹⁾	<5	4	—
7. Coumarin plasma	<1	¹⁾	20	3	+
8. Coumarin plasma	8.5	¹⁾	22	1	+
9. Citrated coumarin plasma Al(OH) ₃ adsorbed	<1	¹⁾	<5	1	—
10. Oxalated coumarin plasma BaSO ₄ adsorbed	<1	¹⁾	<5	1	—
11. Normal oxalated plasma BaSO ₄ adsorbed	<1	<5	<5	1	—
12. B-plasma + phenprocoumon	<1	¹⁾	<5	1	—

1) experimental sample.

Discussion

Oral anticoagulants take effect not only by depressing coagulation factors II, VII, IX, and X, due to an anti-vitamin-K action, but also cause the appearance of a protein called PIVKA (*Protein Induced by Vitamin K Absence*) (5). In prothrombin time methods using thromboplastins prepared from brain from various species (9), this protein exerts a competitive inhibitory effect. The site of inhibition in the reaction sequence of coagulation has been localized at the conversion of factor X from a pro-enzyme into an active moiety (4).

It is thought that the protein in question is a precursor of one or more of the coagulation factors of the prothrombin complex. During the blockade of the vitamin-K-dependent step assumed to be responsible for conversion of the precursor into a coagulation factor (II, VII, IX, or X), synthesis of the precursor in hepatic cells leads to overflow of the precursor into the circulation. On the basis of this hypothesis coagulation-factor like protein could be expected to circulate in vitamin-K-deficiency or oral anticoagulation. In attempting to further define the precursor, Ganrot and Niléhn (2) demonstrated a prothrombin in coumarin plasma with a electrophoretic mobility differing from the normal. They also reported defective adsorption of PIVKA onto BaSO₄, but this finding seems doubtful, because they used citrated plasma and adsorbed with 10 mg BaSO₄/ml. Under these conditions, the adsorption of both the prothrombin complex and PIVKA is known to be incomplete.

Another indication of the presence of a prothrombin-like material in anticoagulated human plasma is the finding of material that develops thrombin activity at a rate much more slowly than prothrombin itself (6). Investigations by both Prentice (10) and Prydz (11) clearly demonstrated an excess of immunologically detectable factor X over factor X activity in anticoagulated samples.

The investigation reported here shows that a significant amount of PTC-inhibitor neutralizing material can be demonstrated in anticoagulated human plasma. The few selected samples used in this study contain very low factor IX activity and thus enabled us to make economic use of the PTC-inhibitor. Higher levels of factor IX activity make the interpretation of the difference from the residual factor IX activity reflecting the amount of PTC-inhibitor neutralizing material progressively more difficult (and therefore create a statistical problem).

The existence of protein induced by vitamin K absence or antagonist has been demonstrated in different ways: as an inhibitor of prothrombin time type estimation, as an abnormal prothrombin, as anti-factor-II cross-reacting material, as antifactor-X cross-reacting material, and, now as PTC-inhibitor neutralizing capacity. We do not as yet want to assume the existence of a different precursor for each of the vitamin K dependent coagulation factors, because the activities mentioned might all be due to a single type of molecule. The possibility that more than one type of PIVKA exists must be taken into consideration.

Summary

By means of the PTC-inhibitor neutralizing test (12) a significant excess of PTC (factor IX)-like material over factor IX activity could be demonstrated in human coumarin plasma. This cross-reacting material is considered to be a precursor of one or all of the clotting factors of the prothrombin complex.

Résumé

A l'aide du test de neutralisation du PTC (12) on met en évidence, dans le plasma d'individus traités aux dérivés de la coumarine, un excès marqué de matériel semblable au PTC (facteur IX) par rapport au facteur IX procoagulant. Ce matériel interférant est considéré comme étant un précurseur d'un ou de tous les facteurs du complexe de la prothrombine.

Zusammenfassung

Mit Hilfe des PTC-Inhibitorneutralisationstestes (12) konnte im menschlichen Coumarin-Plasma ein signifikanter Überschuß an PTC-(Faktor IX)-ähnlichem Material gegenüber der Gerinnungsfaktor-IX-Aktivität nachgewiesen werden. Es wird angenommen, daß dieses kreuzreagierende Material eine Vorstufe eines oder aller Gerinnungsfaktoren des Prothrombinkomplexes ist.

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